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PRINCIPAL INVESTIGATOR: Donald B. Kimmel, DDS, Ph.D.

CONTRACTING ORGANIZATION: Creighton University
Omaha, Nebraska 68131

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Donald B. Kimmel, DDS, Ph.D.

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Creighton University
Omaha, Nebraska 68131

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Principal Investigator's Signature

Date

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Introduction

Stress fractures during Basic Training (BT) occur in 0.5-2% in men to 6-12% in women [1-4], in the US Army. Incidence is higher in Caucasians than in blacks [5], peaking during the second-third weeks, then occurring at a lower, but steady rate for the remainder of BT [6-7]. 75% of stress fractures are of the tibia and foot bones [8-10], producing morbidity requiring care, but usually cured by a few weeks restricted duty. Femur and pelvis fractures are less frequent [11-14], but tend to heal more slowly, and can result in medical discharge, occasionally incurring long-term Army obligation for service-related disability.

Identifying individuals at high risk of stress fracture during BT by a rapid, non-invasive, risk-free measurement, perhaps before their entry into the military (a "4-F skeleton" found at a MEPS), would benefit both female soldiers and the operation of the US Army. It would decrease the Army's: 1) risk of unintentionally injuring soldiers, 2) person-hours lost from duty, 3) commitment to training predictably unfit soldiers, and 4) resources expended caring for injured personnel. A successful stress fracture risk prediction program could lead not only to a less injury prone corps of Army women, but also to a male stress fracture risk prediction program and an understanding of risk factors that can be influenced by altered BT procedures.

A. Parallels of Stress Fracture and Osteoporotic Fracture

Osteoporotic and stress fractures share many characteristics (See Table 1). Thus, stress fracture risk prediction for the military and osteoporotic fracture risk evaluation in the elderly are likely to be similar tasks, meaning that similar equipment might evaluate intrinsic risk in both. Each type of fracture occurs during activities that most participants complete *without* fracture, making the term "fragility" fractures appropriate for both. This simply implies the existence of a skeleton unable to endure loads tolerated well by a "normal" skeleton. Osteoporotic fracture risk has been successfully evaluated during the past decade [15-19]. It is reasonable to think

Table 1
Parallels of Osteoporotic and Stress Fracture

Osteoporotic Fx	Stress Fx
-bones too weak to sustain reasonable, intended uses	-bones too weak to sustain reasonable, *assigned* uses?
-occurs mainly in elderly women	-occurs in soldiers and athletes
-limited to a minority of elderly women	-limited to a minority of soldiers and athletes
-site-specific profile (hip, vertebrae, wrist,)	-site-specific profile (foot bones, tibia, femur, pubic ramus)
-sex preference (female)	-sex preference (female)

that applying similar screening instrumentation to military recruits would successfully evaluate their stress fracture risk.

1. Known Risk Factors for Stress Fracture

The risk for stress fracture during basic training is ~5X higher in women than men [1-4, 21]. Poor physical fitness, previous fracture [6, 8, 22], low femoral neck bone mineral density [23] and small bone size are known risk factors [24-26]. In women, osteoporosis risk factors, like amenorrhea, family history of osteoporosis, and smoking, seem to play a role [27]. Harsh training conditions have occasionally been implicated [28] and protective footwear can reduce stress fracture incidence [29]. Though modifying BT procedures to achieve comparable fitness with fewer stress fractures (delay of pre-identified "injurious" activities (e.g. lengthy road marches, heavy pack/weapon carrying) is reasonable, intrinsic risk factors of recruits need study [30].

2. Measurements of Risk

a. Densitometry

The most commonly measured intrinsic risk factor for osteoporotic persons is osteopenia, low bone mass. This is known because of the existence of excellent radiation-based densitometric techniques for measuring bone mineral density (BMD) *in vivo*. Lifestyle risk factor assessment adds little information to quantitative bone measurements. Since both osteoporotic and stress fracture populations suffer fragility fractures, one could reasonably expect lower bone mass in stress fracture subjects than in non-stress-fracture subjects. More importantly, 10-15 year prospective studies have proven that bone mass measurement predicts risk of not only osteoporotic fracture [15-19], but also *all* fractures [20]. For example, 39% of sixth decade women with normal spinal or wrist BMD eventually develop a fragility fracture. This percentage changes 5% for every .01 g/cm^2 of BMD (10% of population standard deviation). Thus, a 50yo woman with BMD .05 g/cm^2 *above* the population mean has only ~1/7 chance (14%) of developing osteoporotic fracture; a woman with BMD 0.1 g/cm^2 *below* the population mean has a 9/10 chance (89%). We will collect data to allow developing a similar risk prediction algorithm for young female Army recruits.

b. Other Bone Evaluation Techniques

Other bone evaluation techniques predict risk for osteoporosis as well as densitometry, but are better suited for screening, because they are quicker, more economical, and radiation-free. The best example is quantitative ultrasound (QUS) [31]. QUS may work because osteoporosis is characterized by low bone *strength*, a problem caused in practice not only by low BMD, but also by poor bone geometry [32]. For this

reason, osteoporosis investigators now implicate other factors, including poor trabecular structure [32] and fatigue damage [33-34], that seem to be measured by QUS [35]. Bone measurements involving ultrasound transmission and attenuation have thus evolved [36-40]. QUS, done quickly with portable instrumentation, prospectively and retrospectively identifies subjects at increased risk for osteoporotic fracture [41-47] with *no* radiation exposure. It is thus suitable for risk-free screening of large numbers of personnel.

C. Relationship of Fatigue Damage to Stress Fracture

Fatigue damage exists in repetitively-loaded bone, as it does in most materials subjected to repetitive loading [48-50]. It seems likely to predispose to fracture by decreasing bone stiffness [51]. Military BT may be a fatigue loading situation (e.g., 80-90 miles of marching and running in formation during eight weeks) that causes stress fractures. Certainly, association, if not causation, is implied.

The role of fatigue damage in stress fracture has been poorly studied, in equal part due to a lack of animal models and methodology for identifying fatigue damage. One purpose for bone remodeling in adult humans may be to repair fatigue damage [52-54]. Osteoporotic subjects often suffer from a low bone formation (remodeling) rate [55-58], suggesting that their skeletal fragility might be due to slow repair fatigue of damage. Little is known of remodeling rates in stress fracture soldiers, but similar mechanisms could exist. A low turnover animal model might enable better evaluation of the biologic mechanism by which the skeleton compensates for accumulattion of fatigue damage. For example, if fatigue loading in a low turnover animal produces weakness at the usual rate, one must conclude that remodeling plays little role in the development of fatigue-related bone weakness. If fatigue loading in a low turnover animal produces no weakness, one could well conclude that remodeling, *not* microdamage, is the key in the development of fatigue-related bone weakness.

D. Animal Models

1. Externally Applied Loads

Animal models of controlled force application are the best hope for studying fatigue loading effects in the skeleton, because conditions (stress and strain distributions) can be specified, repetitive loading machines can be attached, and experimental conditions can be standardized among animals and experiments. Animal studies can compliment the clinical evaluation of risk by allowing both histomorphometric studies and bone strength evaluation.

Two types of *in vivo* mechanical loading models of external force application with the potential for controlled fatigue loading are currently available: through implants in large animals [59-62], or through muscle and overlying tissue in small animals [63-**Error! Bookmark not defined.**]. The pin implant models provide information, but two disadvantages, the requirement for major surgery likely to affect bone homeostasis [61, **Error! Bookmark not defined.**] and the permanent disruption of gait and normal daily loading conditions, limit them. Non-surgical external force application works through pads overlying soft tissues [63-68]. The advantages of noninvasive force application are: 1) lack of surgical intervention and periosteal disruption, 2) presence of normal cage activity with measured loads superimposed upon normal daily loading patterns, and 3) use of the rat, a mammalian model with a long history of extensive metabolic, endocrine, and skeletal research.

E. Gaps in Knowledge

Both osteoporotic fracture and stress fracture during military BT are fragility fractures. Considerable expertise for evaluating osteoporotic fracture risk exists that can be applied to stress fracture risk prediction. The prospective evaluation of fragility fracture can be done with quantitative ultrasound (QUS). Stress fracture risk prediction actually seems easy when compared to what has been done for osteoporotic fracture risk prediction. Prospective osteoporotic fracture risk evaluation studies for vertebrae involve 10^{4+} person-years of observation. Hip fracture with its lower incidence requires ~$5X10^4$ person-years of observation. Prospective stress fracture risk studies should be limited to the eight week BT period. The logistics of recruiting free-living elderly populations and maintaining them on study (usually in the face of severe illness and death) are frightening. For this proposal, the logistics of recruiting a military pre-stress fracture population have been overcome by obtaining the approval of appropriate Army personnel at the Reception Station and Troop Medical Clinic at Fort Leonard Wood, MO, a BT facility for >5000 women/year.

The relationship of fatigue damage to stress fracture development remains unclear. Since stress fracture arises in fatigue loading situations, such studies can potentially assist in gaining a fundamental understanding of the induction of stress fracture. They can only be undertaken in animal models. Thus, this proposal combines the practical advance of proven risk prediction methodology in a clinical study, with controlled loading that can further the fundamental understanding of the interplay of skeletal conditions with fatigue loading, in an animal study.

Body of Report

A. Hypotheses

1. Clinical

A) Two subsets of female Army recruits with stress fracture during BT exist, one marked by low bone strength and the other by ineffective bone remodeling.

1) In one subset, prospective quantitative ultrasound (QUS) measurements of the calcaneus and medical history risk factors (e.g., amenorrhea, past fracture, poor fitness), individually, or in combination, predict stress fracture risk.

Directly as a result of this study, we expect that QUS measurements coudl become a method by which Army medical personnel can safely screen for new recruits at high risk for stress fracture.

2) In a second subset, QUS values are lower at the time of stress fracture than in time-matched non-fracturing controls.

This study may lead to other methods of prospectively screening for recruits at unacceptably high risk for stress fracture (bone biomarkers).

B) In female Army recruits without stress fractures, QUS values decline in the calcaneus during BT.

We expect this study to reveal a new non-invasive method for detecting bone changes after intense physical activity.

2. Basic

A) Both QUS and bone strength decline in tibiae of female rats exposed to *in vivo* fatigue loading. Histologic signs of fatigue damage are more frequent with increased fatigue loading.

We expect this experiment to reveal incremental fatigue damage in bone and validate our ability to detect it.

B) The healing response to fatigue damage is a combination of accelerated intracortical remodeling, accelerated periosteal bone deposition, and production of endocortical microcallus, that peaks at six weeks and ends by twelve weeks after last fatigue loading.

We expect this study to reveal the time-course of fatigue damage repair. While the results may not apply to the human skeleton, because of basic differences in intracortical remodeling of the rat and human skeletons, the results will be unique and may lead to building new paradigms.

C) Common metabolic conditions of the female skeleton, like estrogen depletion, low turnover, and high turnover influence the usual skeletal healing response after fatigue damage. Estrogen depletion and low turnover slow the response, while high turnover speeds it.

We expect this study to reveal new knowledge about the influence of pre-existing skeletal turnover levels on the response to fatigue damage.

D. Research Design- Clinical Phase I

Clinical Phase I consists of two stages (See Table 2).

Table 2 Clinical Phase I Experimental Design			
Stage	Timing	Procedures	Location
I	Before BT	QUS & Questionnaire	Reception Station
IIA	At Time of SFx	QUS	Troop Medical Clinic
IIB	After BT	QUS	Troop Medical Clinic
BT- Basic Training; SFx- Stress Fracture			

We established a Bone Measurement Center in the Reception Station at Fort Leonard Wood, MO on 25 August 1995. It was equipped with three QUS devices (UBA575+; Hologic Corporation; Waltham, MA) and three full-time staff, a coordinator and two assistants. Two UBA575+'s were in the Reception Station; the third was in the Troop Medical Clinic. We trained the three staff members in the proper use of UBA575+ at the Bone Measurement Center. The team measured a maximum of 50-60 female soldiers per day during weeks of peak flow (1 June-15 August). The coordinator interfaced with Major Mary Laurin, MPT, who diagnosed stress fracture using a stepwise decision tree agreed to by several BT facilities (See *Stress Fracture Diagnosis* Below). The data were analyzed using logistic regression with stress fracture as dependent variable and QUS values and other risk factors as independent variables.

Measurements

Stage I

First, as the female recruits finished blood draws and other administrative duties pursuant to joining the Army during their two days at the Reception Station, we measured QUS in the non-dominant calcaneus in 93% of female recruits presenting during an eleven month period (N=~4350). We also administered a bone risk factors questionnaire. This portion of the project went ~50% faster than projected, because the flow of female soldiers was ~6000/yr, rather than the 4500/yr we had expected. The missed 7% were either on days of extremely high flow (>60/d) or unavailable during the two day measuring window. QUS evaluation caused minimal disruption to the flow of recruits through the Reception Station, due mainly to the cooperation of Colonel Mark Collins and his staff. As possible, we recorded fitness as the total points scored on the fitness test given to the recruits at the beginning of BT.

Stage IIA

At the time of stress fracture, we obtained repeat QUS values in each fracturing recruit and two members of her company that best matched her for age and baseline QUS values.

Stage IIB

At the close of BT, we performed repeat QUS on the calcaneus of 175 randomly chosen recruits who completed BT without stress fracture.

Stress Fracture Diagnosis

This algorithm was followed routinely during the first year of the study when Major Laurin was the chief Physical Therapist. She left Fort Leonard Wood in June, 1996. After that time, because of financial considerations, the diagnosis of stress fracture was done less rigorously.

Stress fracture in soldiers reporting to the Troop Medical Clinic (TMC) with a chief complaint of lower extremity pain, was diagnosed with uniformly-applied clinical/ radiographic criteria generally in use at all Army BT facilities. At the initial visit, "point tenderness" in the lower extremity or pelvis was established through examination of the painful area by an experienced clinician. Trainees with only generalized soreness returned to active BT with no diagnosis. Symptomatic soldiers were placed on limited duty. After seven days, symptom-free recruits returned to active BT with no diagnosis, while symptomatic soldiers remained on limited duty and returned to the TMC after an additional fourteen days. Soldiers symptom-free at the third visit returned to active BT with no diagnosis. All symptomatic soldiers remained on limited duty; lateral and AP radiographs of the painful region were taken. If stress fracture was visualized (by a healing response), a diagnosis of stress fracture was made. Symptomatic soldiers with normal radiographs returned after seven more days, for more radiographs. If stress fracture was present, the diagnosis was made. If the second set of radiographs was normal, a ^{99}Tm scan of the affected region was made. If the scan was focally active, a diagnosis of stress fracture was made. If the scan was not focally active, no diagnosis was made. Each soldier symptomatic at the fourth visit was followed clinically, returning to active BT at a time consistent with her rate of recovery.

Statistical Approach

Finally, we used logistic regression and Cox proportional hazards testing to calculate risk of stress fracture, using SOS (speed of sound), BUA (broadband ultrasound attenuation), and clinical history.

D. Research Results

1. Clinical Phase I

Baseline characteristics of the women measured in Phase I are in Table 3. They represent a typical cross-section of 1995-96 female Army recruits. Of this group, 327 (8.7%) suffered one or more stress fractures at an average of 22±6 days into BT.

The principal risk factors for stress fracture for these women were age and QUS measurement. Though SOS (speed of sound) and BUA (broadband ultrasound attenuation) were correlated (r^2=0.37; P<0.0001), SOS yielded somewhat higher risk coefficients than did BUA. Testing SOS and BUA in the same model showed that they were not independent fracture risk predictors, indicating that they measure similar bone properties underlying fracture risk. Greater age was a minor, independent predictor of fracture risk. Black soldiers were less than half as likely to have stress fracture as whites and Hispanics (4.7% vs. 10.2%). A positive smoking history was also an independent risk factor in this group, suggesting that it was a marker rather than a physiologic cause. When adjusted for race and age, the relative risks accorded to SOS and BUA are in Table 4.

Table 3
Baseline Data for Phase I Females

Number	4234 women
Age	20.9±3.6 yrs
Interval	25 August 1995-15 July 1996
Height	164.1±6.8 cm
Weight	61.1±8.9 kg
BMI	22.7±2.8 kg/m^2
Race	50% White 9% Hispanic 35% Black 6% Other
SOS	1515.2±9.0 m/sec
BUA	100.1±19.0 dB/MHz

Table 4
Relative Risk of Stress Fracture in All Female Soldiers Attributed to QUS Measurements

	unadjusted		adjusted for age and race	
Variable	**Relative Risk**	**Confidence Interval**	**Relative Risk**	**Confidence Interval**
SOS (m/sec)	1.99*	1.76-2.26	1.80*	1.57-2.07
BUA (dB/MHz)	1.73*	1.54-1.94	1.62*	1.39-1.81
PT Score	2.48	1.56-3.94	-	-
Age (yrs)	1.09+	1.06-1.12	-	-

*per standard deviation; +per year of age

Subgroup Analysis

Poor physical fitness, as determined by the PT (Physical Training) Score at entrance into BT, is a predictor of stress fracture. We collected PT Scores on 791 young women from the above group. We then did backward logistic regression, with all of the above factors plus PT Score. PT Score had a higher relative risk, but it was significantly different from the other two significant factors, smoking history and SOS (Table 5). More importantly, when placed in the same

Table 5
Interplay of PT Score and SOS in Phase I

Factor	Relative Risk	Confidence Interval
PT Score	2.60*	1.65-4.10
SOS	1.90+	1.23-4.67
Smoking	2.39	1.28-2.82

adjusted for race and age
*increase in risk per SD decrease below mean

model, both PT Score and SOS were significant predictors of stress fracture, indicating a need to screen for both.

Summary

Like osteoporotic fracture, stress fracture is a fragility fracture whose risk can be assessed prospectively by a simple non-radiation-based bone measurement. Furthermore, the risk assessed by bone measurement is independent of the Army's previous best prospective indicator of stress fracture risk, physical fitness assessment at the start of BT. A low bone QUS value is thus unrelated to poor physical fitness.

From a practical standpoint, however, QUS measurement fails to provide a way of pinpointing individual soldiers with such a high risk of stress fracture that they can be immediately excluded from the Army as is often done for asthma or poor eyesight that creates a certain risk of periodic physical incapacitation and risk to fellow soldiers.

2. Clinical Phase II

In completing Clinical Phase I, the first female phase of screening, we satisfied the work proposed in our grant application. However, with about one year remaining in our time period, we asked for and were granted permission to proceed to Clinical Phase II, the male screening phase. Assuming the same predictive ability for QUS seen in Phase I (RR=~1.8) with a stress fracture incidence of 1%, we calculated we would need to measure ~4500 male soldiers [72]. That seemed possible before the 1 August 1997 termination of the grant. As the male recruits were processed, we measured QUS in the non-dominant calcaneus between 15 July 1996 and 1 May 1997. As possible (N=1454), we again recorded fitness as initial PT Score.

Table 6
Baseline Data for Males

Number	4711 men
Age	21.2±3.2 yrs
Interval	15 July 1996-1 May 1997
Height	178.0±7.3 cm
Weight	76.8±12.6 kg
Race	63% White 12% Hispanic 19% Black 6% Other
SOS	1519.7±11.4 m/sec
PTScore	135.7±51.8
BUA	96.8±7.9 dB/MHz

Baseline characteristics of the males (N=4711) are in Table 5. Of this group, 11 (0.23%) suffered a stress fracture, an insufficient number to analyze for relative risk of fracture *for any factor*. It is of note that BUA in these 11 was 87.2±16.5 dB/MHz (vs. 96.8±7.9 dB/MHz [Table 5]) and SOS was 1516.3±19.9 m/sec (vs. 1519.7±11.4 m/sec [Table 5]). This degree of difference in QUS values of fracturing vs. non-fracturing soldiers (Table 5) was similar to that in females, suggesting that QUS values differed in fracturing males and non-fracturing males, but that our study undersampled, creating a Type II error.

Three significant changes in BT occurred at Ft Leonard Wood around 1 July 1996. First, the directors of BT at began to introduce strenuous activities at more gradual pace during BT. Second, Major Laurin left Fort Leonard Wood for advanced schooling at Fort Leavenworth, KS. Third, since all soldiers with stress reaction type injury are treated similarly, the formal diagnosis of stress fracture makes little difference. Thus, optimal resource management at the TMC dictated that fewer radiographs be taken of symptomatic soliders who may have had stress fractures.

It can be readily appreciated how each change reduces the chance of diagnosing stress fracture. The change in BT strategy seems likely to decrease stress fracture by reducing demands on recruits, making it partially responsible for the lower than expected incidence of stress fractures in our male soldiers. The departure of Major Laurin and the execution of fewer radiographs at the TMC, combined with the practical focus on simply returning soldiers to training, rather than making a diagnosis, also would tend to reduce the number of stress fractures diagnosed.

3. Clinical Phase III

We completed Clinical Phase II on about May 1, 1997. Army personnel at Ft Leonard Wood wondered if their changes in BT routines had reduced stress fracture incidence, due to the more gradual conditioning that was possible with the more gradual ramp-up to longer marches with heavy packs and weapons. They felt that if this were true, conditioning might no longer be a risk factor, but that QUS values would continue to be a risk factor. We asked for permission to extend our work to include measuring another 2100 female recruits.

To test this, we requipped our Bone Measurement Center in the Reception Station with a new QUS device (Sahara; Hologic Corporation; Waltham, MA). The new device was six times faster than the previous UBA575+'s. We calculated with a relative risk of 1.8, and a stress fracture incidence of 3%, we would need to measure around 2100 female recruits [72]. We measured 2401 female recruits between 15 June 1997 and 15 October 1997. Only 35 were diagnosed with stress fracture (1.4%), about one-sixth the number from the study done in 1995-1996. However, ~325 developed stress reactions requiring a visit to the TMC. Baseline characteristics are

Table 7
Baseline Data for Phase III Females

Number	2401 women
Age	19.8±3.1 yrs
Interval	15 June 1997-15 October 1997
Height	163.8±7.7 cm
Weight	60.3±8.7 kg
Race	56% White 10% Hispanic 28% Black 6% Other
SOS	1577±48 m/sec
PTScore	84.3±60.6 (N=1524)
BUA	83.1±17.0 dB/MHz

given in Table 8. Note the different SOS and BUA values that are derived from the new instrument. Studies showed that the Sahara had about a correlation of about 0.7 for SOS and BUA, but with substantially different y-intercepts. The data were analyzed using logistic regression with stress fracture as dependent variable and QUS values as independent variables.

Table 8
Relative Risk of Stress Fracture in All Female Soldiers Attributed to QUS Measurements

	unadjusted		adjusted for fitness	
Variable	Relative Risk	Confidence Interval	Relative Risk	Confidence Interval
SOS (m/sec)	1.47*	1.02-2.13	1.35	0.86-2.12
BUA (dB/MHz)	1.49*	1.02-2.18	1.38	0.98-2.15
Fitness	1.81*	1.13-2.88	-	-
Age (yrs)	1.08+	1.05-1.11	-	-

*per standard deviation; +per year of age

As with the 1995-1996 soldiers, QUS value remained a significant risk factor for stress fracture. However, a number of background circumstances changed in two years. The BT regime had been adjusted to be less demanding. The diagnosis of stress fracture was now more likely to be missed because of fewer radiographs and Major Laurin, who had paid close attention to diagnoses, was gone. We also collected PT Scores on about twice as many female recruits as previously, so chances are better that we have a better idea of the relationship of QUS and PTScore. Only 35 recruits were diagnosed with stress fracture (1.4%), about one-sixth the incidence from the 1995-1996 study. However, ~325 developed stress reactions requiring a visit to the TMC. The relative risks for all factors were less than previously. Though one cannot be sure whether BT adjustments or decreased ascertainment were the cause, it is reasonable to believe that stress fracture incidence has declined.

1. Basic Phase

a. *In Vivo* Loading Model and Experimental Design

The purpose here was to test skeletal responses to fatigue loading in low, normal, and high turnover skeletons. First, 83 6 month old intact female Sprague-Dawley rats were randomly divided into six groups for necropsy at -4, 0, 3, 6, 9, and 12 weeks from the last session of fatigue loading. The lower right leg was externally loaded *in vivo* in a four-point bending device on Monday, Wednesday, and Friday of each week for four consecutive weeks, a total of twelve loading sessions per rat (24,000 cycles). Fatigue loading in four point bending (2000 cycles/day, 2Hz @41N (2200-2500με at periosteal

and 1800με at endocortical surface by finite element modeling). The left leg was not externally loaded, serving as a control. During each loading session, rats were maintained under light ether anesthesia. Between loading sessions, rats returned to normal cage activity. The lower pads (loading points) of the device are 23mm apart and contact the medial leg surface, while the upper pads are 11mm apart and contact the lateral leg surface. Uniform strain is created at the lateral bone surface between the upper pads (Figure 1). This creates a region of the tibia 3.5-14mm proximal to the distalmost aspect of the tibia-fibular junction (TFJ), that is the region of concentrated fatigue loading. Force is applied from the lateral side of the leg by a motor driven, programmable, four-point bending apparatus, at 45-50N@2Hz, for 2000 cycles/d.

Intraperitoneal (IP) injections of calcein (8 mg/kg; Sigma, St. Louis, MO) were given on a schedule of 1d ON, 6d OFF, 1d ON, and 2d OFF (1-6-1-2) before sacrifice. At the time of tissue collection, all rats were anesthetized by IP injection of Ketamine (50 mg/kg body wt) and Xylazine (10 mg/kg). Death was induced by intracardiac injection of .25cc of Fatal Plus (Vortech Pharmaceuticals, Dearborn, MI). Right and left tibiae from each group were processed for histomorphometric analysis and *ex vivo* ultrasound evaluation.

b. Fatigue Damage Detection

1. Quantitative Ultrasound (QUS)

To quantitate fatigue damage, speed of sound (transmission mode) was measured by quantitative ultrasound (QUS) in the longitudinal axis of the right tibia using 1Mhz transducers (Panametrics, Waltham, MA). Comparison between right (loaded) and left (unloaded) tibiae was made with the Kruskal-Wallis test ($P<0.05$) and intergroup testing by Student Neuman-Keuls ($P<.05$).

2. Basic Fuchsin Block Staining

The loaded region of each tibia at 1-1.5cm proximal to the TFJ was immersed in 1% basic fuchsin [69] and allowed to dry. The bone was embedded as above and sectioned at 70μm, then examined for the presence of stained and unstained cracks in the tissue. Stained cracks are presumed to have been present before the sectioning procedure.

c. Histomorphometry

All bones were cleaned of non-adherent muscle and soft tissue, and placed in 70% EtOH. For histomorphometry, the region of the tibial diaphysis extending from the TFJ to 1cm proximal to the TFJ was cut and placed in Villanueva stain [70] for 72hrs and then returned to 70% EtOH. Over the next 14 days, the specimens were dehydrated in

graded ethanols, defatted in acetone, and embedded in modified methyl methacrylate [71]. The samples were cross-sectioned at 70μm with a 0.6mm inter-section distance on a saw-microtome (Model 1600, Leitz Instruments; Deerfield, IL USA).

Two sections in the region 5.5-9.0 mm proximal to the TFJ were mounted on slides, given a random number, and analyzed semi-automatically with a light/epifluorescent microscope and a camera projecting an image onto a screen connected to a Windows NT computer with appropriate software (BIOQUANT True Color for Windows (R&M Biometrics, Nashville, TN)). For counting, the cross section was divided into lateral tibia periosteum, medial tibia periosteum, tibial endosteum, and fibula periosteum (Figure 2). The following data were collected at the specified magnifications in each region: at 20X- cortical bone and medullary area; at 40X- bone surface (Ps.BS and Es.BS); at 100X- double and single-labeled calcein surface, double-labeled calcein surface, and woven bone surface (no surfaces fell into more than one category); and at 400X- interlabel width (IrL.Wi) at sites of double labeling. The following calculations are made for each region: periosteal woven bone area (PWo.Ar (%)), periosteal new lamellar bone area (PNLmB.Ar), endocortical new lamellar bone area (ENLmB.Ar), periosteal mineralizing surface (PMS/BS), and endocortical mineralizing surface (EMS/BS).

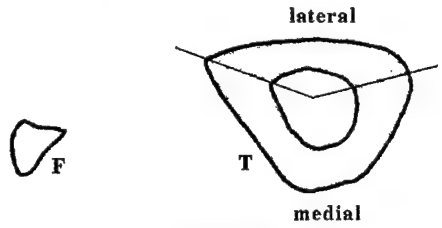

Figure 2
Cross-Section through Loaded Region
F-fibula; T-tibia; lateral and medial periosteum regions are indicated. The endosteum was considered as one unit.

Table 9
Bone Response to Fatigue Loading in Adult Female Rats

	-4 Wk	0 Wk	3 Wk	6 Wk	9 Wk	12 Wk
PWo.Ar*, %	0.0±0.0	2.78[a]±2.5	11.4[a]±17.6	0.0[b]±0.0	0.0[b]±0.0	0.0[b]±0.0
PNLmB.Ar, %	0.0±0.0	1.59±1.38	1.89±1.99	4.44±5.41	2.94±8.10	3.20±2.86
ENLmB.Ar*, %	0.0±0.0	0.39±0.68	2.98±2.70	7.10[ab]±1.21	5.89[ab]±5.23	0.0±0.0
PMS/BS, %	0.3±0.2	4.1±11.1	4.1±6.6	5.7±3.2	9.1±7.0	35.5±51.8
EMS/BS*, %	4.2±2.3	21.9[a]±15.4	35.5[a]±10.0	33.4[a]±15.2	10.2[a]±11.9	5.1[b]±1.1
QUS*, m/sec	3805±114	3738±171	3734±119	3790±84	3845[b]±67	3781±75

mean±SD
* Kruskal-Wallis (P<.05)
[a] difference due to fatigue loading compared to -4wk P< .05
[b] difference due to fatigue loading compared to -0 wk P< .05

In intact rats, no fractures occurred. No differences in total bone area existed. All histologic variables (Table 9) were obtained by subtracting left leg values from right leg

(loaded) values. Periosteal, but not endocortical, woven bone was present only at the end of the fatigue loading period and three weeks later. Significant endocortical accumulation of new lamellar bone was present at six and nine weeks after the end of fatigue loading, but was no longer apparent at twelve weeks. This change was mirrored by the significant trends in endocortical mineralizing surface. QUS was lower at Weeks 0-3 than at baseline, then returned to baseline by Week 6. Basic fuchsin staining revealed no quantitative or qualitative differences among the groups.

b. *In Vivo* Loading in Ovariectomized Rats

Ovariectomy induces high turnover in the skeleton, particularly accelerating formation at periosteal surfaces. 50 6 month old female Sprague-Dawley rats were ovariectomized from a dorsal approach. Ten weeks later, they were randomized into five groups for necropsy at -4, 0, 4, 8, and 12 weeks from the last session of fatigue loading. Loading as above was completed during the next four weeks. At autopsy both tibiae were collected for histomorphometric evaluation of periosteal formation surface (PFS), mineral apposition rate (MAR) and woven bone surface (WoBS).

Table 10a
Bone Response To Fatigue Loading In Ovariectomized Rats

Endpoint	Leg	-4 Wk	0 Wk	4 Wk	8 Wk	12 Wk	P[a]
pFS, %	Right	36±6	70[b]±18	27[b]±4	16±3	13±3	.0001
	Left	37±5	21.0±25	14±4	7±3	7±3	.0001
pMAR, µm/d	Right	1.3±0.1	1.9[b]±0.4	1.6[b]±0.2	1.1[b]±0.2	0.7±0.1	.0001
	Left	1.3±0.1	0.8±0.2	0.9±0.1	0.8±0.1	0.8±0.1	.007
pWoBS, %	Right	0.0±0.0	56[b]±16	6.7[b]±6	1.3±4	0.1±0.4	.0001

Table 10b
Bone Response To Fatigue Loading during Estrogen Treatment

pFS, %	Right	-	50[b]±6	14±3	5±2	13[b]±0.4	.0001
	Left		28±5	9±4	1.1±0.4	3.4±0.2	.0001
pMAR, µm/d	Right	-	1.6[b]±0.2	2.2[b]±0.1	0.9±0.1	1.0±0.1	.NS
	Left		1.1±0.2	1.5±0.1	0.8±0.1	0.7±0.1	NS
pWoBS %	Right	-	58[b]±12	11±4	0±0	2.6±1.2	.0001
TotAr, mm^2	Right	-	6.3±0.2	5.5±0.3	5.4±0.3	5.7[b]±0.3	NS
	Left		5.9±0.1	6.0±0.2	5.9±0.2	6.2±0.3	NS
NBAr, mm^2	Right	-	.035[b]±.012	.09[b]±.03	.064[b]±.015	0.13±.012	.02
	Left		.001±.001	.013±.012	0±0	0±0	NS

mean±SEM, [b] difference within each group (Left [C] vs Right [L] leg) P< .05

Periosteal mineral apposition rate was transiently increased by fatigue loading up to 8 weeks after cessation of loading, but then returned to values seen on the control side. Similarly, formation surface was much higher on the loaded leg than on the

control side up to four weeks after cessation of loading. The loading response appears to be of similar timing, but more marked in ovariectomized rats than in intact rats above (compare to Table 9).

c. *In Vivo* Loading in Estrogen-Treated Intact Rats

Estrogen treatment suppresses bone turnover, particularly at periosteal surfaces. It is a reasonal substitute for antiresorptives like bisphosphonates. 40 6 month old female Sprague-Dawley rats were treated with 17-ß-estradiol (10µg/kg in corn oil 2X/wk) for two weeks, a dose that blocks bone loss in newly ovariectomized rats. Then, as treatment continued, they were fatigue-loaded as above for four weeks, and killed at 0, 4, 8, and 12 weeks from the last session of fatigue loading. At autopsy, both tibiae were collected for histomorphometric evaluation of periosteal formation surface (PFS), mineral apposition rate (MAR) and woven bone surface (WoBS).

No fractures were observed. There were no differences in total bone area among groups. Periosteal formation surface was significantly greater in the loaded legs of the Week 0 group than at any other time (Table 10b). Periosteal mineral apposition rate (pMAR) was significantly greater due to loading at Weeks 0-4. Periosteal, but not endocortical, woven bone in the right loaded leg was present only at 0-4 weeks after the end of the fatigue loading period. All periosteal woven bone had finished forming by Week 8. New bone area (NBArR) was significantly greater in loaded (right) legs at 0-8 weeks post fatigue loading. Consistent with greater periosteal than endosteal strains in vivo fatigue loading causes a greater periosteal than endocortical response.

Summary

In vivo fatigue loading causes a robust, but transient periosteal and endocortical response. The endocortical response is more marked and long lasting, producing new lamellar bone that is incorporated into the existing bone in an indistinguishable fashion by 12 weeks after cessation of fatigue loading. The endocortical surface response of bone formation is as expected considering the bone accumulation pattern. The periosteal response adds only woven bone that disappears by six weeks after cessation of fatigue loading. The results in ovariectomized and estrogen-treated rats suggest that the skeletal response to fatigue loading is somewhat greater in rats with a higher turnover rate.

The trend in QUS, a measurement that can detect fatigue damage in many materials, is compatible with a burden of fatigue damage that increases then declines. The basic fuchsin staining studies failed to find any trend that would support the QUS

trends. We regard this dichotomy as methodologic and suggest the fatigue damage is really present.

While it was definitely possible to perform fatigue loading in these rats, it is clear that the findings are far from definitive. Though we detected no fractures, the periosteal and endosteal woven bone responses are rather similar to what is seen during the healing of human stress fractures, including raised periosteum and localized transient woven bone. It is likely that, in the absence of detectable fracture, the response in the loaded region is a typical periosteal stress reaction. We are relatively certain that we did not induce fatigue fracture in this experiment, and suggest that either longer-term loading or mildly more intense loading would be necessary. Our pilot work showed that 2000 cycles per loading session (@2Hz) was our maximum because of damage to the overlying soft tissue. It is possible that other types of apparati that can deliver loading cycles at 10-30Hz might be more successful in inducing fatigue damage in vivo. We never saw Haversian remodeling induced in the cortical bone of rats in response to fatigue loading.

Conclusions

Clinical

The most important new conclusion of this report is that Army recruits with weak bones are more likely to have stress fractures during Basic Training. Recruits with weak bones can be identified propsectively by the same instrumentation used to quantitate osteoporotic fracture risk. We confirmed that less physically fit soldiers are more likely to have stress fracture. Recruits with poorer fitness tend to have weak bones. There are usually elements of self-selection by stronger persons for a lifestyle that results in better conditioning involved in this. We also confirmed that sex, age, race, and past smoking history are measurable risk factors. It seems likely that the Army could very efficiently: 1)measure fitness, 2) measure QUS, 3) record age, 4) query on smoking history. If they could stratify companies of persons with fitness and QUS one standard deviation below normal and over age 25 with a history of smoking, and give such persons a twelve week BT with a very gradual increase in activities, the Army would see reductions in stress fracture.

Basic

The most marked response to measured fatigue loading in small animals is woven bone formation at the periosteal surface. The excess woven bone subsides within four weeks of stopping fatigue loading. No fatigue damage could be detected and

confirmed with certainty. It appeared that skeletons of higher turnover mountained a somewhat more vigorous response to fatigue loading.

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